Early Life Conditions and Adolescent Sexual Orientation: A Prospective Birth Cohort Study

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Abstract

This study tested the association between multiple prenatal and postnatal early life factors and adolescent sexual orientation in a longitudinal birth cohort. Factors included birthweight, gestational age, parental ages at birth, number of older brothers and sisters, breastfeeding, maternal anxiety/depression, family socioeconomic position, parent-child relationships, parental absences, pubertal body mass index, and housing issues. We used data on 5007 youth from the Avon Longitudinal Study of Parents and Children (ALSPAC). Sexual orientation was assessed using a 5-point scale of sexual attraction at 15.5 years. Early life factors were separated into three developmental periods: prenatal (n = 9), before 7 years (n = 5), and after 7 years (n = 5). We controlled for childhood gender nonconformity (GNCB), handedness, and digit ratio as markers of prenatal androgen exposure. GNCB was strongly associated with later male and female nonheterosexuality, and higher right-hand digit ratio was associated with later male nonheterosexuality. Boys with low birthweight and shorter breastfeeding duration were more likely to have a later nonheterosexual orientation. Boys with parental absence before 7 years of age were more likely to be nonheterosexual, but this effect disappeared after entering all early life history factors. Parental absence since birth, low prenatal family socioeconomic position, and poorer parent-child relationship were associated with later nonheterosexuality among girls. The results are discussed in the context of a life history framework for understanding human sexual orientation development in males and females.

Keywords: Life history; sexual orientation; homosexuality; birthweight; gender nonconformity; ALSPAC

Human sexual orientation is likely to be multifactorial in its origins. Biological and psychosocial factors may influence the lifecourse of sexual orientation (affecting sexual attractions, identity, and sexual behaviors differently; Bailey et al., 2016). This is consistent with multifactorial influences on other developmental traits such as personality. However, scholars often imply multiple influences on sexual orientation but rarely, if ever, test them. Research suggests that genetic factors explains approximately one-third of the variation in sexual orientation (Bailey et al., 2016). Thus, most of the differences between people in their sexual orientation are due to environmental factors (often non-shared) pointing to multiple etiology. Causal pathways are rarely tested in prior research because of the use of cross-sectional designs, surveys of adult heterosexuals and nonheterosexuals which are susceptible to reporting biases, and problems accounting for dependencies in data (Bailey et al., 2016). Few datasets lend themselves to testing a range of biological and psychosocial variables. We therefore examined the role of multiple prenatal and postnatal early life factors on the development of adolescent sexual orientation in a longitudinal, birth cohort from England.

Life History Theory

Life history theory aims to explain the diversity in life trajectories of organisms, especially in their reproductive histories (Del Giudice, Gangestad, & Kaplan, 2015). It can be conceived of as a "biopsychosocial" model, integrating genetic and non-genetic (e.g., socialization, environment, and learning) processes in accounting for variation in development and reproduction. In humans this framework has focused on the influence of early life conditions (those characterized by high mortality and morbidity, environmental unpredictability, low parental investment, and resource scarcity) on sexual behavior traits across the lifespan. Prior research suggests that humans will adopt "fast" or "slow" sexual life history strategies according to the early environment they experience (Ellis, 2004). The fastness or slowness is often defined by the age at which reproductive or sexual behaviors

begin. For example, studies have reported associations between low birthweight, or other indicators of reduced parental investment during the early years (such as father absence), and accelerated pubertal maturation among human females (Quinlan, 2003). Various explanatory models propose that differences in early life experiences may influence subsequent sexual behavior by constraining an individual's growth or by acting as forecasts of ecological conditions (Ellis, 2004). This makes responding to variable early life conditions with changes in sexual behavior a facultative, adaptive response. While much research has focused on female sexual life history, one meta-analysis revealed that low family socioeconomic position (SEP), maternal absence, and paternal absence were associated with early sexual debut, early first birth, and early marriage among men (Xu, Norton, & Rahman, 2018).

Life History Theory and Sexual Orientation

Life history theory is generally silent on the trait of sexual orientation. We have argued previously that life history approaches are relevant to sexual orientation (Xu et al., 2018). We hypothesize that heterosexuality is an optimal and adaptive (in fitness terms) life history strategy given the species-typical pattern of sexual interests in humans is towards the opposite sex. Nonheterosexuality represents a departure from that generally fitness-enhancing pattern of sexual behavior. Nonheterosexuality (homosexuality/ bisexuality) may represent a "fast" life history or be a by-product of other life history traits. Epidemiological research suggests that people with infrequent homosexual behavior and attractions are more common than those with substantial or exclusive homosexuality (Gates, 2011). This may permit some direct reproduction to occur because heterosexual contacts still dominate in the former group, and help transmit alleles related to nonheterosexuality in a population. A broader phenotype of "nonheterosexuality" may comprise reproductive and non-reproductive sexual contacts.

A life history approach also has the potential to integrate biological and psychosocial accounts of the development of human sexual orientation. The dominant theories regarding

sexual orientation development are biological. For example, prenatal androgen theory proposes that high prenatal androgen exposure during critical periods may be related to heterosexuality in men and homosexuality in women, whereas low androgen exposure may be associated with homosexuality in men and heterosexuality in women (Ellis & Ames, 1987). Another model, the maternal immunity hypothesis, proposes that carrying successive male fetuses triggers a maternal immune response that feminizes brain development and results in nonheterosexuality in later born sons (Blanchard, 2018; Bogaert et al., 2018).

Twin studies have shown that sexual orientation has a modest heritability (about 30%; Alanko et al., 2010). However, the twin concordance rate for sharing the same sexual orientation is estimated as 24% across men and women (Bailey et al., 2016). A large proportion of monozygotic twins who are nonheterosexual have heterosexual co-twins (approximately 76%). As these twins share the same genotype, prenatal and early postnatal environment factors must play a large role in their different sexual orientations. The environmental influences appear non-shared rather than shared (Bailey et al., 2016). We have previously proposed that early life conditions suggested by life history theory may constitute one source of these non-shared factors in the development of male sexual orientation (Xu et al., 2018). They may also interact with the proposed biological mechanisms. For example, prenatal early life factors (e.g., poor maternal condition) may alter hormonal processes during fetal development, which then bias subsequent brain sex differentiation affecting circuitry related to sexual orientation (Rahman, 2005). Alternatively, early hormonal processes may themselves constitute a third factor which links early life factors and later sexual orientation. Several candidate early life factors may be important in sexual orientation development.

Birthweight

Variation in birthweight may serve as an indicator of adverse early environments or maternal condition and predict later sexual life history strategy. Low birthweight is associated

with earlier first pregnancy in women (and mediated by SEP; Nettle, Coall, & Dickins, 2011). In general, low birthweight offspring of both sexes may attract less parental investment. Birthweight may also be associated with male sexual orientation and interact with sibling sex composition. Specifically, it is suggested that maternal immune response triggered by carrying successive male fetuses results in nonheterosexuality and low birthweight in later born sons (Blanchard & Ellis, 2001), but studies are inconsistent. One study reported that homosexual men with older brothers had lower birthweights than their heterosexual counterparts (Blanchard & Ellis, 2001). Another study found no difference in birthweights between feminine and control boys with fewer than two older brothers (Blanchard et al., 2002). Blanchard (2012) also proposed that mothers of firstborn homosexual sons were more likely to develop an immune response to a fetus which may lower their birthweight (see also Bogaert et al., 2018). Again, results are inconsistent (Skorska, Blanchard, VanderLaan, Zucker, & Bogaert, 2017). It is also not clear why mothers of firstborn homosexual men without younger siblings would more likely to develop an immune response.

Older Brothers

The number of older brothers is robustly associated with male sexual orientation (Blanchard, 2018). Homosexual men are usually born after their siblings, and this late position in birth order is associated with the number of older brothers but not with the number of older sisters, younger sisters, or younger brothers (Blanchard, 2018). This phenomenon has been termed the fraternal birth order effect or FBO. Here, each male fetus the mother carries appears to increase the odds of homosexuality in later born males by about 33% (see Blanchard, 2018). It has been hypothesized that carrying successive male fetuses triggers a maternal immune response (possibly towards Y-linked minor histocompatibility antigens from male fetuses), and this feminizes the brain development of later born males increasing the odds of expressing a homosexual orientation (Bogaert et al., 2018). However, FBO

cannot explain the sexual orientation of all nonheterosexual men and studies thus far are cross-sectional.

Parental Involvement and Psychosocial Factors

Low parental investment characterized by parental absence, shorter breastfeeding duration, and other forms of psychosocial hardships, appear to be associated with fast reproductive life histories in women and men (Nettle et al., 2011; Xu et al., 2018). For example, paternal absence before 5 years of age is associated with earlier menarche in girls (Quinlan, 2003 cf. Sear, Sheppard, & Coall, 2018; Sohn, 2017). Again, few studies are longitudinal in design and associations are generally weak. While the suggestion of psychosocial or family influences in reproductive life history is widely studied, its role in sexual orientation is socially and scientifically controversial. The history of research in this area has tended to see psychosocial explanations as more stigmatizing of nonheterosexuality than biological ones (Bailey et al., 2016). However, theories of causation may not be directly relevant to specific social and moral positions. Monozygotic twin differences clearly point to non-shared environmental influences. This contributes to the complexity of sexual orientation development, comprising both biological and non-biological influences. The role of social factors is likely to be small based on the existing evidence, with few studies of good methodological quality (Bailey et al., 2016). Importantly, the role of environmental factors is hypothesized to be stronger in female than male sexual orientation given its greater malleability. Women report more bisexuality, report greater change in sexual identity and behavior patterns over time, and have a sexual orientation that is less category-specific compared to men (Bailey et al., 2016). The hypothesis of greater environmental involvement in female sexual orientation is thus uncontroversial.

Proposed psychosocial factors have predominantly included parent-child relationships. Poorer parent-child relationships may be indicators of low parental investment and thus be

associated with nonheterosexuality. Bell, Weinberg, & Hammersmith (1981) found weak correlations between retrospective ratings of parent-child relationship traits and sexual orientation, but this effect became either nonsignificant or weak when childhood gender nonconformity was controlled. This highlights important problems in testing psychosocial factors. Nonheterosexuality may precede poor parent-child relationship. Firstly as prehomosexual children are likely to be gender nonconforming, this may negatively influence relationships with parents (Kane, 2006). Homosexual men may also show elevated rates of separation anxiety in childhood and this could also place negative pressure on parent-child relationships (VanderLaan, Gothreau, Bartlett, & Vasey, 2011). The parents of pre-homosexual children may also differ from parents of pre-heterosexual children in ways that influence parent-child relations. Strong evidence against parental psychosocial influences comes from studies of children raised by nonheterosexual parents. These show no differences in sexual orientation between children reared by nonheterosexual parents and those reared by heterosexual parents (see Bailey et al., 2016). However, these studies rely on small convenience samples and may be confounded by third factors, such as genetic parental effects. There is no robust work examining early familial SEP or other forms of parental investment on the development of sexual orientation to our knowledge. Nonheterosexual youth are more likely to experience early disruption such as homelessness but it not clear whether this is related to family environment or other social factors such as discrimination and stigma (Gattis, 2009). Studies using longitudinal designs may be better able to test the causal role of these and confounding factors (such as childhood gender nonconformity).

Other Early Life Factors

Prior studies have found a range of other indicators of adverse early life conditions including preterm birth, having older parents, shorter duration of breastfeeding, and maternal anxiety/depression, are associated with faster sexual life history strategies (James, Ellis,

Schlomer, & Garber, 2012; Wehkalampi et al., 2011). Such factors may also be associated with later sexual orientation but are poorly studied. Often they interact with each other making associations with outcomes difficult to disentangle. Older maternal age has been reported to be associated with nonheterosexuality (Xu et al., 2018) or homosexual marriage in men (Frisch & Hviid, 2006), but results are inconsistent (e.g., Blanchard & Bogaert, 1996).

Developmental Sensitive Periods

Several developmental stages may be important sensitive periods for the influences of early life conditions and their impact upon later sexuality. Life history scholars have suggested that the first five or seven years of life may be the most important (Simpson, Griskevicius, Kuo, Sung, & Collins, 2012). This may be due to pre-pubertal hormonal changes including adrenarche, the 5-to-7 years old shift in cognition, language, and social skills; the responsivity of developing neural systems to adverse environments; and increased self-sufficiency during this time (Ellis, 2004). Middle childhood (6 to 11 years) may also be important, especially since sex differences in physiology (strength and muscularity), social behavior, and aggression intensify during this stage (Del Giudice, 2014).

Biological models of sexual orientation development (e.g., prenatal androgen) suggest that prenatal periods are critical (Rahman, 2005). However, there may be more than one critical period for males, and more sensitive periods for females, during which sex hormones act (McCarthy, Herold K, & Stockman, 2018). Psychosocial approaches to sexual orientation are silent on which developmental periods are important. This lack of specificity is a weakness of social explanations of sexual orientation. Given the role of developmental stages in life history and in models of sexual orientation, the present study investigated early life factors in prenatal, postnatal before 7 years, postnatal after 7 years periods.

Childhood Gender Nonconforming Behavior and Prenatal Hormonal Exposure

Three important developmental correlates of sexual orientation require comment here. Nonheterosexual men and women report more childhood gender-nonconforming behavior (GNCB), on average, than heterosexual adults. This pattern has been found in both prospective and retrospective studies (Bailey & Zucker, 1995; Li, Kung, & Hines, 2017). Digit ratio (2D:4D) is a marker ascribed to the actions of prenatal androgens. This ratio shows a moderate sex difference (smaller in men than women; Hönekopp & Watson, 2010). Women with congenital adrenal hyperplasia, a condition characterized by high prenatal androgen exposure, exhibit smaller (more masculine) digit ratios (Brown, Hines, Fane, & Breedlove, 2002). Nonheterosexual women have more masculine digit ratios than heterosexual women but there is no difference in digit ratios between heterosexual and nonheterosexual men (Grimbos, Dawood, Burriss, Zucker, & Puts, 2010). Finally, handedness is a somatic trait that appears robustly associated with sexual orientation. Nonheterosexual men and women are significantly more likely to be non-right-handed than heterosexual men and women (Lalumiere, Blanchard, & Zucker, 2000).

The role of these variables in the life history framework is speculative but offer plausible hypotheses. Early life factors could promote GNCB which then cascades into later nonheterosexuality (because early life factors are causally closer to GNCB than to sexual orientation). Or GNCB, handedness, and digit ratio could have independent influences on both early life conditions and sexual orientation. Early life conditions may alter prenatal androgen exposure, and then influence later sexual orientation. Or, GNCB could act as a behavioral proxy for a common underlying mechanism, such as prenatal androgen exposure (with handedness and digit ratio acting as more direct markers; Skorska & Bogaert, 2017). As prenatal androgen theory is the dominant model of sexual orientation development, the present study examined these three indicators of androgen exposure as potential covariates.

The Present Study

Here we use data from a prospective birth cohort in England to test whether a range of early life factors (in prenatal, pre-7 years of age, and post-7 years developmental stages) were associated with adolescent sexual orientation at age 15.5 years. It appears to be appropriate to begin measuring sexual orientation at 15.5 years old. Studies also show that men and women recall first having feelings of sexual attraction at approximately age 10, on average (McClintock & Herdt, 1996). One study reported a mean age of self-reported first awareness of same-sex attraction at approximately 15 years (Calzo, Antonucci, Mays, & Cochran, 2011). Some studies report even earlier recalled mean age of awareness of same-sex attractions (e.g., Floyd & Bakeman, 2006). Changes in reported sexual orientation identity were also found to occur at a similar rate throughout adolescence and into emerging adulthood (Ott et al., 2011). This is the first study of its kind and includes a range of early life factors never studied before in relation to sexual orientation. The longitudinal design will permit better tests of causal pathways. Here we test the extent to which prenatal factors and postnatal early life factors predict later sexual orientation in boys and girls separately. We hypothesized that prenatal early life factors (e.g., low birthweight) would be associated with nonheterosexuality in both boys and girls, and postnatal early life factors (e.g., poorer parent-child relationship) would be associated with nonheterosexuality in girls since their sexual orientation is more socially influenced. We further controlled for the influences of GNCB, handedness, and digit ratio in our analyses.

Method

Participants

Participants were part of the Avon Longitudinal Study of Parents and Children (ALSPAC). All pregnant women with an expected date of delivery between 1st April 1991 and 31th December 1992 in the Bristol area of the South West of UK were eligible and invited to attend the ALSPAC. The initial sample recruited 14,541 (71.81% of the eligible

sample) pregnant women who delivered 14,062 live-born children and 13,988 were alive at 1 year. Additional recruitment attempting to bolster the original sample with eligible cases who had failed to join the study at the beginning resulted in 15,458 fetuses with data collected from the age of seven onwards. Of this total sample of 15,458 fetuses, 14,775 were live births and 14,701 were alive at 1 year of age. Fifty-nine percent of the cohort attended the "Teen Focus" sessions and have been followed four times between the age of 12.5 years old and 17 years old. For more details, see Boyd et al. (2013). The study website contains details of all the data, which are available through a searchable data dictionary: http://www.bris.ac.uk/alspac/ researchers/data-access/data-dictionary/. Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees, and King's College London Psychiatry, Nursing & Midwifery research ethics subcommittee (protocol reference number: LRS-16/17-4194 and name of the study: testing a life history approach to the study of variation in human sexual orientation). We analyzed ALSPAC data reported by parents and children across different time points. Adolescents who reported a valid response of sexual orientation and sexual behavior (see Supplemental Text 1) at 15.5 years old were included here, N = 5,007 (2,349 boys and 2,658 girls).

Measures

Sexual orientation. At 15.5 years old, adolescents were required to answer the question: "Please choose the description that best fits how you think about yourself" on a 5-point Kinsey-like scale: 1 = *100% heterosexual*, 2 = *mostly heterosexual but also attracted to the same sex*, 3 = *bisexual*, 4 = *mostly homosexual but also attracted to the opposite sex*, 5 = *100% homosexual*, 6 = *not sexually attracted to either sex*, and 7 = *not sure*. This was done via computer to promote disclosure of sensitive personal information. Adolescents who chose "*not sexually attracted to either sex*" (n = 17) or "*not sure*" (n = 91) were excluded from the

analyses. This is because we had no a priori predictions about the role of early life conditions in adolescents with ambiguous or no sexual attractions and many such adolescents identify as heterosexual later in life (Ott, Corliss, Wypij, Rosario, & Austin, 2011; Savin-Williams & Joyner, 2014). Such 5-point scales of sexual attractions have been used in large studies of adolescents (Austin et al., 2009; Ott et al., 2011; Remafedi, Resnick, Blum, & Harris, 1992; Saewyc, Skay, Bearinger, Blum, & Resnick, 1998). Saewyc et al (1998) also pilot tested their items with a youth sample before full-scale implementation. The 5-point measures show good stability (i.e., test-retest over 2 year intervals) in adolescents (Ott et al., 2011), expected associations with sex of sexual partners among adolescents (Remafedi et al., 1992; Saewyc et al., 1998), and low nonresponse rates compared with measures of other components of adolescent sexual orientation (Saewyc et al., 2004). As bisexuals may differ from gay/lesbian individuals in some components of sexual orientation, we treated them as separate groups. Accordingly, adolescents who chose *100% heterosexual* or *mostly heterosexual but also attracted to the same sex* were coded as heterosexual, those who chose *bisexual* were coded as bisexual, and those who chose *mostly homosexual but also attracted to the opposite sex* or *100% homosexual* were coded as homosexual. As a result, 2,290 heterosexual boys (45.73%), 29 bisexual boys (0.58%), 30 homosexual boys (0.60%), 2,585 heterosexual girls (51.63%), 56 bisexual girls (1.12%), and 17 homosexual girls (0.34%) were included.

Body size. Birthweights (kgs) were taken from birth notification and/or obstetric data and/or recorded by ALSPAC measurers in the delivery room. For data recorded by more than one method, we used the following criteria. If birthweight values from each measurement method were identical, then the value was accepted; if disagreement between birthweight values from different measurement methods were less than 100 grams, then the lowest value was accepted; if disagreement between birthweight values from different measurement methods were greater than 100 grams, then the value was coded as missing (no

observations in our sample had disagreement greater than 100 grams). At 14 years and 7 months old adolescents gave their height and weight, to generate body mass index (BMI).

Gestational age. This was based on the date of mother's last menstrual period, pediatric or obstetric assessment, and ultrasound assessment. Adolescents were categorized into three gestational age groups: preterm birth (< 37 weeks' gestational age), term birth (37 - 41 weeks' gestational age), and postterm birth (> 41 weeks' gestational age) (Savitz et al., 2002).

Parental age. Maternal age was recorded as the age at the last menstrual period. When adolescents were at 12 weeks' gestation, the partner of the mother was required to report whether he was the father of the child. If he reported "yes", his age at completion of questionnaire was coded as paternal age; otherwise, paternal age was coded as missing.

Maternal anxiety and depression. When adolescents were 18 weeks' gestational age and 8 weeks old, two subscales of Crown-Crisp Experiential Index (CCEI) were used to measure maternal anxiety and depression (Ross & Hafner, 1990). These have acceptable reliability and validity (Alderman, Mackay, Lucas, Spry, & Bell, 1983; Burgess, Mazzocco, & Campbell, 1987). The test-retest reliabilities over a year period were .77 and .72 for anxiety and depression, respectively (Crown, Duncan, & Howell, 1970). Patients diagnosed with anxiety disorder and depression scored very highly on corresponding CCEI subscales (Crisp, Jones, & Slater, 1978). Each subscale consists of 8 items, rated on a 4-point scale from 1 = *never* to 4 = *very often*. An example item is "Do you worry a lot". We recoded this into four variables: prenatal maternal anxiety/depression and postnatal maternal anxiety/depression. Since prenatal maternal anxiety and depression were correlated (r = .77), the average of prenatal maternal anxiety and depression was used in the analysis. We did similarly for postnatal maternal anxiety and depression (r = .73).

Older siblings. At 6 months, adolescents' mothers reported the numbers of older brothers and older sisters who live with the adolescents, including maternal and paternal half-brothers and half-sisters, step brothers and step-sisters, fostered children, and adopted children.

Family structure changes. At six different points in time, adolescents' mothers answered the questions: "Is the present live-in father-figure/mother-figure the biological father/mother of the study child?" and "How old was the child when the biological father/mother stopped living with the child?". Father absence and mother absence were recoded into two variables (father absence and mother absence) with four categories: never with father/mother, father/mother absence before 7 years of age, father/mother absence since 7 years of age, and father/mother present. Due to low rates of mother absence, it was necessary to combine these variables to indicate parental absence (see missing data section).

Duration of breastfeeding. When adolescents were 1 years and 3 months old, their mothers reported whether their children were breast-fed (*yes* or *no*) and the duration of breastfeeding in months. Duration of breastfeeding in months was used in the analysis, and adolescents who were not breast-fed received a score of 0 on this variable. Maternal reports of breastfeeding initiation and duration appear accurate and reliable (Li, Scanlon, & Serdula, 2005).

House moves. At six different points in time, adolescents' mothers reported how many times they have moved home since last interview. We recoded this information into two variables: the number of house move before 7 years of age, and since 7 years of age.

Parent-child relationship. When adolescents were 9 years 7 months old, they rated their relationship with their parents on a 5- point scale ranging from 1 = *not true* to 5 = *true*. Nine items developed by the ALSPAC study team were used. An example item is "I have a parent who I have a lot of fun with". Cronbach's alpha for the scale in our sample was .82. Exploratory factor analysis yielded one factor (eigenvalue = 3.78) accounting for 42.03% of

the variance in our sample (Supplemental Table 1). The total score of the nine items was used in the analysis, with a higher score indicating a better relationship with parents.

Family socioeconomic position (SEP). Family SEP was assessed via parents' education, parents' occupation, household income, and family financial difficulties. When adolescents were 32 weeks' gestation old, their mothers reported their own and their partner's highest educational qualifications (*CSE, vocational, O level, A level, and Bachelor's Degree*).

At six different points in time, adolescents' mothers reported their own occupation. At five different points in time, the mothers' partners reported their own occupation. The Standard Occupational Classification (2000) was used to categorize occupation type. We recoded this into four variables, mother's lowest occupation before adolescents were born, father's lowest occupation before adolescents were born, mother's lowest occupation before adolescents were 7 years old, and father's lowest occupation before adolescents were 7 years.

At four different points in time, adolescents' mothers also answered the question: "On average, about how much is the take-home family income each weak?" Participants were required to choose from *Less than £100*, £*100 - £199, £200 - £299, £300 - £399, £400 or more*, and *Don't know*. This was recoded into two variables, lowest family income before adolescents were born, and lowest family income before adolescents were 7 years old.

At six different points in time, adolescents' mothers answered the question: "How difficult at the moment do you find it to afford these items? (e.g., food, clothing, and heating)" on a 4-point scale from 0 = *not difficult* to 3 = *very difficult*. Five items were used to measure financial difficulties. This was recoded into three variables: the worst financial difficulties before adolescents were born, the worst financial difficulties before adolescents were 7 years old, and the worst financial difficulties since adolescents were 7 years old.

Since these indicators of family SEP are correlated (polychoric correlation from .19 to .63), summary scores incorporating these indicators were constructed: prenatal family SEP,

family SEP before 7 years of age, and family SEP since 7 years of age. We applied polychoric principal component analysis, and used the loadings on the first principal component as item weightings to generate a summary score for each developmental stage. A higher score indicates lower family SEP (Supplemental Table 2 for factor loadings). The first component explained 49.20%, 50.27%, and 55.32% of the variation in prenatal family SEP, family SEP before 7 years of age, and family SEP since 7 years of age, respectively.

Childhood gender-nonconforming behavior. When adolescents were 2 years 6 months, 3 years 6 months, and 4 years 9 months old, mothers rated their children's gender-nonconforming behaviors (GNCB) using the Preschool Activities Inventory (PSAI) (Golombok & Rust, 1993). PSAI is a validated self-report questionnaire (Golombok & Rust, 1993). PSAI also has acceptable reliability (test-retest reliability over a year = .64; split-half reliability = .88) (Golombok & Rust, 1993). The PSAI consists of 12 male-typical items and 12 female-typical items assessing children's toy preferences (e.g., jewellery), activity preferences (e.g., playing at fighting), and characteristics (e.g., avoid getting dirty). Each item was rated using a 5-point scale ranging from 1 = *never* to 5 = *very often*. The PSAI is scored via deducting the total score for female-typical items from the total score for the male-typical items, then transforming to a pseudo-*T* scale by multiplication with 1.10 and adding 48.25 (Golombok & Rust, 1993). A higher score indicates more male-typical behavior and less female-typical behavior for both girls and boys. The average of the three time points was used (GNCB at these three times significantly and consistently predicts adolescent sexual orientation; Li, Kung, & Hines, 2017).

2D:4D digit ratio. When adolescents were 11 years old, photocopies of their hands were taken. They were required to place the ventral surface of both hands flat onto the photocopier, and the lengths of the second and the fourth digits for each hand were measured to 0.01mm using the "Mahr digital caliper16 EX" (from tip of finger to basal crease). This method has

been shown to be accurate and reliable (Ribeiro, Neave, Morais, & Manning, 2016). The digit ratio (2D:4D) was calculated as the ratio of the lengths of the second digit to the fourth digit.

Handedness. At 9 year 7 months, adolescents were asked which hand they prefer to use for 6 activities (e.g., "Which hand to you draw") rated from 1 = *Left*, *2* = *Either*, 3 = *Right*, and 4 = *Do not do this at all*. Cronbach's alpha for the scale in our sample was .84. Adolescents who chose *Do not do this at all* were coded as missing. Higher total scores indicated greater right-handedness.

Procedure

Missing Data

The variables had 2.78% - 56.80% missing information within the analysis sample (Table 1 and 2). These missing data were handled using multiple imputation stratified by sex. Prior to imputation, we examined the potential missing data mechanisms using logistic regression to assess whether the observed variables predict missingness. The results indicated that the data were unlikely to be missing completely at random (e.g., duration of breastfeeding and house moves before 7 years predicted the missingness of family SEP before 7 years).

For the imputation model, recommendations for longitudinal studies are that all variables in the analysis should be included (White, Royston, & Wood, 2011). Thus, the outcome variable (sexual orientation), predictors (early life conditions), covariates (e.g., GNCB), and an auxiliary variable (sexual behavior; see Supplemental Text 1) that independently related to the outcome were included. Recommendations also instruct that the number of imputations should be at least as large as the percentage of missing data (White et al., 2011). Thus, we used 57 imputations. We used the chained equations algorithm (MICE) model since we have a combination of continuous and categorical variables. The continuous variables included in the current study were not normally distributed (*Shapiro-Francia* test

showed that all *p*s < .001). Consequently, we used predictive mean matching since this approach makes no distributional assumption.

Imputation for mother absence failed to converge due to small cell sizes. Thus, we were forced to combine father absence and mother absence into one variable labelled "parental absence" (never with father or mother, either parent absence before 7 years, either parent absence since 7 years, and both parents presence). Trace plots and other diagnostics provided no cause for concern regarding the imputed values. Typically, sensitivity analysis comparing analyses based complete-case and imputed data would be undertaken, however, due to the proportion of missing data in the sample this was not possible.

Data Analysis

All analyses were performed in Stata 15.0 and carried out separately for boys and girls. First, a univariate ordered logistic regression was estimated with sexual orientation (heterosexual, bisexual or homosexual) regressed onto each early life variable to determine the unadjusted association. Then a three-step hierarchical multivariable ordered logistic regression was estimated with early life factors entered in sequential manner based on the age period at which they were measured and controlling for covariates (GNCB, 2D:4D digit ratio, and handedness). In the first step, prenatal early life factors (e.g., birthweight and gestational age) were entered. In the second step, early life factors before 7 years were entered. In the third step, early life factors since 7 years were entered.

Finally, we used the *mimrgns* command to calculate the average predicted probability of being homosexual for each significant early life factor and covariate in the final model generated by the third step of the multivariable ordered logistic regression. The predicted probabilities for each continuous variable were computed using its 25th/50th/75th percentile and observed values for the remaining variables in the model. We also calculated the predicted probability of being homosexual for adolescents with all the significant factors and

covariates present, and for adolescents with none of these significant factors and covariates. The significant continuous variables were set to the 25th/75th percentile for present (75th for variables with odds ratios greater than 1 and 25th for variables with odds ratios less than 1) and 50th percentile for absent, and the remaining variables were set to the observed values.

The ordered logistic regression analyses assume that the outcome measure is ordinal in nature and that the association between the predictor and outcome is equivalent across the levels of the outcome (*proportional odds*). That is, the log-odds ratio for the predictor in a logistic model where the outcome is heterosexual vs bisexual/homosexual is equivalent to the log-odds ratio for the model where the outcome is heterosexual/bisexual vs homosexual. The Brant test was employed iteratively to assess the likelihood that this assumption held for each predictor (Brant, 1990). Where the test was significant at the 5% level the assumption was relaxed for that predictor. Thus, one odds ratio is presented where the proportional odds assumption held and two are presented where it did not.

Since bisexual and homosexual individuals may differ in some components of sexual orientation, it is possible that the outcome in the ordered regressions is not ordinal in nature. To assess the robustness of our estimates to varying conceptualizations of sexual orientation, we also estimated logistic regression where the outcome was heterosexual vs bisexual/homosexual, and multinomial logistic regression where the outcome was heterosexual vs bisexual and heterosexual vs homosexual. In these models no assumption is made about the outcome being ordinal or the proportionality of the odds (Supplemental Table 3 to 6).

While no formal power calculation was conducted, it is possible to consider power to detect a meaningful effect. At the 5% level, the sample size allows for the detection of odds ratio of greater than approximately 1.8 for a binary predictor variable with 80% power, where

the proportional odds assumption holds for the predictor. As such, despite the low number of nonheterosexuals in the sample, power to detect meaningful effects is acceptable.

Results

Boys

GNCB (odds ratio from 0.888 to 0.902, all *p*s < .001) and right 2D:4D digit ratio (odds ratio from 1.185 to 1.283, all *p*s < .01) were significantly associated with nonheterosexual orientation in both univariate and multivariable regressions, while there were no significant associations between left 2D:4D digit ratio, handedness, and sexual orientation (Table 3 and 4). When the odds ratio was transformed to the percentage change in the ratio for one-unit increase in the predictor using the formula: 100 × (*odd ratio* - 1), the results suggested that boys who displayed more GNCB had 10.90% to 12.60% greater odds of being nonheterosexual, and boys with higher right 2D:4D digit ratio had 18.50% to 28.30% greater odds of being nonheterosexual.

Birthweight (odds ratio from 0.458 to 0.570, all *p*s < .05) and duration of breastfeeding (odds ratio from 0.904 to 0.921, all *p*s < .05) were strong predictors of nonheterosexual orientation in both regression models. Boys with low birthweight had 75.40% to 118.30% greater odds of being nonheterosexual, and boys with shorter duration of breastfeeding had 8.60% to 10.60% greater odds of being nonheterosexual. Greater number of older brothers (odds ratio from 2.069 to 2.254, all *p*s < .05) was significantly associated with homosexual orientation in multivariable regressions, indicating that boys with greater number of older brothers had 106.90% to 125.40% greater odds of being homosexual. Greater number of house moves since 7 and parental absence before 7 years of age were also significantly associated with nonheterosexual orientation in univariate regression, but these disappeared in the multivariable regression when all early life factors were entered into the model.

To further aid understanding of the results, the significant odd ratios (relative difference) from the third step of the multivariable ordered logistic regression were transformed to average marginal effects (absolute difference). Boys who displayed more GNCB (25th percentile) had a 1.73%, 95% confidence interval (CI) = 1.10% - 2.36% probability of being homosexual, boys with higher right 2D:4D digit ratio (75th percentile) had a 1.65%, 95% CI = 1.02% - 2.29% probability of being homosexual, boys with lower birthweight (25th percentile) had a 1.57%, 95% CI = 0.99% - 2.16% probability of being homosexual, and boys with shorter duration of breastfeeding (25th percentile) had a 1.80%, 95% CI = 1.08% - 2.51% probability of being homosexual. Boys with more GNCB, higher right 2D:4D digit ratio, lower birthweight, and shorter duration of breastfeeding had a 3.74%, 95% CI = 2.00% – 5.49%, probability of being homosexual, while boys with none of these had a 0.78%, 95%CI = 0.36% - 1.20% probability of being homosexual (Figure 1).

Girls

Consistent with the results for boys, GNCB was a strong predictor of nonheterosexual orientation among girls in both regression models, with odds ratio ranging from 1.072 to 1.097, all *p*s < .01, indicating that girls who displayed more GNCB had 7.20% to 9.70% greater odds of being nonheterosexual. There were no significant associations between left or right 2D:4D digit ratios, handedness, and sexual orientation (Table 3 and 5).

Low prenatal family SEP (odds ratio ranging from 1.110 to 1.343, all *p*s < .05), parental absence since birth (odds ratio ranging from 2.501 to 4.494, all *p*s < .05), and poorer reported relationship with parents (odds ratio ranging from 0.928 to 0.931, all *p*s < .05) were also significantly associated with nonheterosexuality in both regression models. These indicated that girls with a low prenatal family SEP had 11.00 % to 34.30% greater odds of being nonheterosexual, girls with parental absence since birth had 150.10 % to 349.40% greater

odds of being nonheterosexual, and girls with a more negative relationship with parents had 7.40% to 7.80% greater odds of being nonheterosexual.

In terms of average marginal effects, girls who displayed more GNCB (75th percentile) had a 0.74%, 95% CI = 0.38% - 1.10% probability of being homosexual, girls with low prenatal family SEP (75th percentile) had a 1.24%, 95% CI = 0.35% - 2.12% probability of being homosexual, girls with parental absence before 7 had a 1.35%, 95% CI = 0.35% - 2.35% probability of being homosexual, and girls with poorer reported relationship with parents (25th percentile) had a 0.65%, 95% CI = 0.34% - 0.96% probability of being homosexual. The predicted probability of being homosexual for girls with more GNCB, low prenatal family SEP, parental absence before 7, and poorer reported relationship with parents was 3.36%, 95% CI = 0.03% - 6.69%, while girls with none of these had a 0.29%, 95%CI = 0.09% - 0.49% probability of being homosexual (Figure 2 and 3).

Discussion

This study in a prospective birth cohort produced three main findings. Firstly, boys with low birthweight and shorter duration of breastfeeding were more likely to be nonheterosexual (bisexual and homosexual), and boys with greater number of older brothers were more likely to be homosexual. Secondly, boys with parental absence before 7 were more likely to be nonheterosexual, but this association disappeared after entering all early life factors into the statistical models. Finally, parental absence since birth, low prenatal family SEP, and low parent-child relationship scores predicted nonheterosexual orientation among girls. These results were found while controlling for GNCB, handedness, and 2D:4D digit ratio.

Early Life Conditions and Sexual Orientation in Boys

The findings regarding birthweight and older brothers is consistent with prior work in cross-sectional samples (Blanchard, 2018; Skorska et al., 2017). However, caution must be exercised in interpreting these findings. The number of older brothers in the present cohort

included half-brothers, step brothers, fostered brothers, and adopted brothers. The maternal immunity theory behind the FBO effect predicts that only previously carried biological male siblings should increase the probability of homosexuality of later born male fetuses (Bogaert, 2006). The birthweight findings support growing evidence showing low birthweight predicts faster sexual life history (Nettle et al., 2011). In addition, low birthweight among later nonheterosexuals boys offers support for maternal immunity hypothesis linking birthweight and FBO (although here we find those two effects to be independent; Blanchard, 2012). None of the early life conditions since boys were 7 years were significantly related to sexual orientation in the multivariate regressions, adding further support to the importance of the prenatal or early postnatal developmental period for male sexuality (Xu et al., 2018).

The association between breastfeeding duration and male nonheterosexuality is novel. Low breastfeeding duration may be related to birthweight, although the direction reported here is opposite to what is typically found (low birthweight infants usually receive more breastfeeding as per medical advice). At this stage, the link between breastfeeding and male sexual orientation is unclear but does potentially indicate lower maternal somatic investment in later nonheterosexual boys. Trade-offs underlying breastfeeding decisions (physiological as well as behavioral) by mothers are well documented (Tully & Ball, 2013). Cross-cultural work suggests that economically wealthier mothers will breastfeed sons more frequently than daughters, whereas poorer mothers will breastfeed daughters more frequently than sons (Fujita et al., 2012). This consistent with the prediction that selection pressures favor parental investment in daughters when times are hard and in sons when times are easy. It is not clear how this plasticity in breastfeeding-response during resource rich or poor conditions applies to sexual orientation.

Early Life Conditions and Sexual Orientation in Girls

The pattern of results for girls is consistent with the notion that female sexual orientation is more influenced by environmental factors, perhaps over the life course (rather than restricted to the prenatal or early postnatal period; Baumeister, 2000). This is supported by cross-sectional studies reporting associations between environmental factors and female sexual orientation. These include education level, religion affiliation, peer influence, and parental influences (e.g., parents age and divorce; Baumeister, 2000). Women report more sexual fluidity over the life-course, more bisexuality, and a less category-specific sexual orientation compared to men (Bailey et al., 2016). However, it is not clear why greater female malleability in sexual orientation would be causally related to social factors. Behavioral and identity components of female sexual orientation may fluctuate as a result of situational factors but attractions may remain stable (Diamond, 2008). Other studies have found no associations between changes in same-sex attractions among women and parental divorce, family SES, education level, or family disapproval of one's sexuality (Diamond, 2008).

The present study covaried GNCB, handedness, and digit ratios as proxy markers of prenatal androgen exposure. Thus, the associations found here cannot be explained by GNCB which is often a confounding variable between putative causal factors (such parent-child relationships) and sexual orientation (Bell et al., 1981). Our results replicate the finding that boys and girls who showed more GNCB before 4.75 years old were more likely to be nonheterosexual. This finding is consistent with a large body of work in retrospective and prospective studies (Bailey & Zucker, 1995; Li et al., 2017). In this study, GNCB was used a proxy behavioral marker of androgen exposure. We encourage future studies to test GNCB as a formal mediator variable. This will provide a better test of its role of a third factor explaining the pattern of the results.

We found that boys with higher (more feminine) right digit ratio were more likely to be nonheterosexual later in life, consistent with the prediction that low levels of prenatal

androgens influence male homosexuality. However, this is inconsistent with a meta-analysis showing no differences in digit ratio between heterosexual and nonheterosexual men (Grimbos et al., 2010). That study only found an association between low digit ratio (more masculine) and female nonheterosexuality, which we did not find here. We did not replicate an association between greater non-right-handedness and later nonheterosexuality, which also contradicts prior studies (Lalumiere et al., 2000). Some studies suggest that sexual orientation difference in digit ratio and handedness vary according to criteria used to classify individuals as nonheterosexual (Xu & Zheng, 2016, 2017). This could be one explanation for our results. The longitudinal nature of our design (handedness and digit ratio measured before sexual orientation) may have contributed to the inconsistency. Although it is difficult to see why this would be the case given handedness and digit ratios appear stable over time.

A Life History Perspective

We have suggested that early life conditions documented in life history research may constitute one source of non-shared environmental factors in the development of sexual orientation. Our findings on sexual orientation are consistent with known associations between similarly measured early conditions and a range of other "fast" reproductive and sexual behavior outcomes (James et al., 2012; Xu et al., 2018). Such factors may interact with processes hypothesized by other models of sexual orientation, such as prenatal androgen theory. For example, prenatal early life factors may influence the levels of sex hormones during fetal or early postnatal brain development (Rahman, 2005). The present findings suggest that life history models should pay attention to the specificity of early conditions, developmental stages, and sexual behavior outcomes. The current results also suggest that the experience of early life conditions may operate in a facultative manner depending on sex with potential greater plasticity of responses among females across developmental periods.

In general, the associations between early life conditions and sexual orientation in both boys and girls were small. This may indicate that other causal factors influence both our predictor and outcome variables, including genetic correlations (Barbaro, Boutwell, Barnes, & Shackelford, 2017). Twin research shows a small influence of the shared environment (including familial influences) on a range of behavioral traits, including sexual behavior (Polderman et al., 2015). Twin research on sexual orientation conforms to this pattern, even for female sexual orientation where some studies show small shared environmental influences and others none (Bailey et al., 2016). Thus, the influence of genetic, shared, and non-shared environmental factors may also depend on the type of early life condition, developmental stage and sexual orientation outcome (attractions, behavior, and identity).

Strengths and Limitations

The present study has particular strengths including the use of a prospective design in a well-characterized cohort, with early life factors (and covariates) measured before sexual orientation. This is important because most studies on the development of sexual orientation rely on cross-sectional or correlational designs. Prospective studies reduce the risk of recall biases and get closer to possible causal pathways. Our design also makes the possibility of reverse causation between early life conditions and later sexual orientation less likely. The range of early life conditions has never been tested before and their choice was theoretically and empirically motivated (using a life history perspective and models of sexual orientation development). We also controlled for GNCB, one possible confounding variable between hypothesized causal factors and later sexual orientation (Bailey et al., 2016).

However, several limitations are important to note. There may be unobserved confounders or “third variables” between early life factors and later sexual orientation that affect the results. Although reverse causation is less likely with longitudinal designs, other sources of confounding remain. Such unmeasured variables include personality factors,

parental awareness or suspicions about their child's later sexual orientation (via elevated gender nonconformity), or unmeasured family dynamics impacting upon some of the early life factors (e.g., parental conflict resulting in house moves). These third factors could also include other indicators of a "fast" life history strategy, such as sexual maturation and number of sexual partners which we could not measure here. Importantly, we could not control for genetic confounds such as shared genes between parents and children. Nor could we control for unmeasured genetic and environmental confounds that load simultaneously on early life conditions, our covariates and sexual orientation (Barbaro et al., 2017). This requires further study. Maternal reports of some of the early life factors may either under- or overestimate their prevalence among adolescents who later become nonheterosexual. For example, mothers with gender nonconforming children could engage in less parental investment (indexed by factors such duration of breastfeeding, parent-child relationships and so on) which is then associated with later nonheterosexuality. There is growing evidence that gender nonconforming children experience negative reactions and greater stigmatization from family and peers and so this possibility requires further study.

The nature of the cohort meant that several early life factors could not be simultaneously measured in all three developmental periods. Some of the measures of early life conditions (e.g., family SEP) may also act as mediators between other conditions and later sexual orientation. SEP, owing to its close association with growing up in a resource rich or poor context, requires careful study. As does the link between birthweight and duration of breastfeeding. In addition, of the seventeen early life factors two (parental absence and gestational age) had restricted response categories which may have reduced power. For some of these measures, validity and reliability information was not available.

The sample sizes of nonheterosexual boys and girls were small, especially homosexual girls. The rates for gestational age and parental absence were low and were not observed in

one or more of the groups. Thus, a small increase in the number of nonheterosexual boys/girls who experienced parental absence, or were preterm birth, will result in larger odds ratios. However, given the low prevalence of nonheterosexual orientation among the population, small numbers of nonheterosexuals in longitudinal or other cohort studies are to be expected. Despite the small number of nonheterosexuals, the power to detect meaningful effects was acceptable due to the large overall size of the sample. The use of ordered regression models improves precision gains for the odds ratios, particularly where some groups are small (or where there are many categories) since one can carry over power from the larger groups to the smaller ones. While case-control studies would afford greater power this would come at the cost of associated biases.

The current study measured sexual orientation when adolescents were 15.5 years of age. Prior research found that the number of people who identified themselves as nonheterosexual increases from adolescence to adulthood (Austin et al., 2009). Thus, it is possible that adolescents may change their sexual orientation reports if we reassess our cohort at later ages, which may even produce somewhat different results. Adolescents may also misreport their sexual orientation (Savin-Williams & Joyner, 2014). Future studies must investigate such cohorts at later ages, and model any change in sexual orientation outcomes and their impact on model estimates. The use of a single-item measure of sexual orientation is also a limitation. Future studies should aim to measure several components of sexual orientation (e.g., identity, attractions, and behavior) or more fully explore the reliability and validity of single-item measures over several time points during adolescence and young adulthood.

Conclusion

The results offer support to the hypothesis that early life factors influence sexual orientation in adolescent boys and girls. The developmental stage of these factors appears important to sex differences in adolescent sexual orientation. Among boys, prenatal and early

life conditions before 7 years of age predicted later sexual orientation. Among girls, a mix of factors measured prenatally and later in childhood predicted later sexual orientation. These associations were found while controlling for putative markers of prenatal androgen influence. GNCB was a strong predictor of sexual orientation as previously shown. Note the role of those influences that could also be conceptualized as "psychosocial" appears small across the board. Future longitudinal studies should test for the role of possible third variables (or genetic confounds) which may act of mediators for the associations found here.

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Table 1
Descriptive Statistics for Early Life Conditions and Covariates among Boys.

Variable	Sexual orientation		
	Heterosexual	Bisexual	Homosexual
Prenatal early life factors			
Birthweight (in kilograms)			
N	2159	29	25
M (*SD*)	3.47 (0.59)	3.28 (0.62)	3.20 (0.52)
Gestational age (*N)*			
Preterm birth	131	-	-
Term birth	1887	25	23
Post-term birth	168	-	-
Maternal age (in years)			
N	2186	29	26
M (*SD*)	28.59 (4.55)	27.83 (4.78)	27.85 (3.56)
Paternal age (in years)			
N	1571	21	16
M (*SD*)	31.53 (5.41)	30.29 (6.94)	31.75 (4.88)
Prenatal maternal depression/anxiety[a]			
N	1867	25	24
M (*SD*)	4.50 (2.72)	4.58 (2.69)	5.34 (3.13)
Prenatal family socioeconomic position[b]			
N	1618	22	18
M (*SD*)	4.84 (2.72)	5.21 (2.76)	5.75 (2.43)
Number of older brothers			
N	2138	29	28
M (*SD*)	0.38 (0.62)	0.17 (0.38)	0.50 (0.64)
Number of old sisters			
N	2123	29	27
M (*SD*)	0.36 (0.61)	0.41 (0.68)	0.22 (0.42)
Early life factors before 7 years			
Duration of breastfeeding (in months)			
N	1704	23	20
M (*SD*)	6.07 (4.71)	5.04 (4.87)	3.60 (3.52)
Postnatal maternal depression/anxiety[a]			
N	1989	26	24
M (*SD*)	3.24 (2.78)	3.12 (2.85)	4.17 (3.60)
Number of house moves before adolescents were 7			
N	1498	19	20
M (*SD*)	1.06 (1.44)	1.26 (1.63)	1.20 (1.61)
Family socioeconomic position before adolescents were 7[c]			
N	1007	14	14
M (*SD*)	5.08 (2.70)	5.17 (3.28)	5.01 (2.63)
Early life factors since 7 years			
Family socioeconomic position since adolescents were 7[d]			
N	1588	23	16
M (*SD*)	2.73 (1.98)	3.22 (2.18)	3.07 (2.21)
Parent-child relationship[e]			
N	1790	22	22
M (*SD*)	41.30 (4.46)	41.36 (4.26)	39.86 (7.83)

Variables	Sexual orientation		
	Heterosexual	Bisexual	Homosexual
Early life factors since 7 years			
Pubertal body mass index			
N	1277	13	13
M (*SD*)	20.21 (2.98)	21.29 (4.28)	20.13 (3.89)
Number of house moves since adolescents were 7			
N	1984	26	23
M (*SD*)	0.32 (0.59)	0.62 (0.90)	0.43 (0.59)
Father absence (*N*)			
Never with father	54	-	-
Father absence before adolescents were 7	192	7	-
Father absence since adolescents were 7	64	-	-
Father presence	1706	16	20
Mother absence (*N*)			
Never with mother	-	-	-
Mother absence before adolescents were 7	-	-	-
Mother absence since adolescents were 7	-	-	-
Mother presence	2002	27	23
Covariates			
Childhood gender nonconforming behavior[f]			
N	1706	25	21
M (*SD*)	61.93 (7.28)	55.96 (10.30)	54.64 (7.73)
Left 2D:4D			
N	2128	27	24
M (*SD*)	0.96 (0.03)	0.96 (0.04)	0.97 (0.02)
Right 2D:4D			
N	2125	27	25
M (*SD*)	0.96 (0.03)	0.97 (0.04)	0.99 (0.03)
Handedness			
N	1882	23	22
M (*SD*)	15.85 (3.13)	16.48 (1.95)	16.00 (2.79)

Note: "-" means 5 or less. Cell counts 5 or less are not presented in order to comply with ALSPAC publication requirements.

[a]The range is from 0 to 16.

[b]The range is from 0 to 15.32.

[c]The range is from 0 to 16.40.

[d]The range is from 0 to 12.25.

[e]The range is from 9 to 45.

[f]The range is from -4.55 to 101.05.

Table 2

Descriptive Statistics for Early Life Conditions and Covariates among Girls.

Variable	Sexual orientation		
	Heterosexual	Bisexual	Homosexual
Prenatal early life factors			
Birthweight (in kilograms)			
N	2423	53	16
M (*SD*)	3.37 (0.50)	3.43 (0.44)	3.43 (0.42)
Gestational age (*N)*			
Preterm birth	96	-	-
Term birth	2185	49	15
Post-term birth	178	-	-
Maternal age (in years)			
N	2459	53	16
M (*SD*)	28.37 (4.52)	28.92 (5.94)	28.06 (4.17)
Paternal age (in years)			
N	1758	37	10
M (*SD*)	31.22 (5.41)	31.65 (5.77)	32.20 (5.25)
Prenatal maternal depression/anxiety[a]			
N	2042	40	11
M (*SD*)	4.50 (2.74)	5.22 (3.09)	4.66 (2.20)
Prenatal family socioeconomic position[b]			
N	1824	35	11
M (*SD*)	4.93 (2.73)	5.60 (2.73)	6.66 (3.05)
Number of older brothers			
N	2391	49	16
M (*SD*)	0.38 (0.63)	0.31 (0.62)	0.63 (0.81)
Number of old sisters			
N	2395	49	16
M (*SD*)	0.37 (0.60)	0.33 (0.72)	0.31 (0.60)
Early life factors before 7 years			
Duration of breastfeeding (in months)			
N	1859	41	11
M (*SD*)	6.36 (4.75)	6.85 (4.94)	8.36 (6.22)
Postnatal maternal depression/anxiety[a]			
N	2287	43	15
M (*SD*)	3.27 (2.74)	3.91 (3.53)	3.00 (2.01)
Number of house moves before adolescents were 7			
N	1637	31	8
M (*SD*)	1.14 (1.47)	1.26 (1.71)	1.63 (1.51)
Family socioeconomic position before adolescents were 7[c]			
N	1101	20	7
M (*SD*)	5.21 (2.80)	5.46 (2.49)	5.43 (2.13)
Early life factors since 7 years			
Family socioeconomic position since adolescents were 7[d]			
N	1745	31	10
M (*SD*)	2.84 (2.04)	2.98 (1.97)	3.02 (1.64)
Parent-child relationship[e]			
N	2085	34	14
M (*SD*)	42.22 (3.77)	40.82 (4.79)	39.79 (4.73)

Variable	Sexual orientation		
	Heterosexual	Bisexual	Homosexual
Early life factors since 7 years			
Pubertal body mass index			
N	1334	21	6
M (*SD*)	20.83 (3.36)	22.26 (3.90)	22.27 (5.04)
Number of house moves since adolescents were 7			
N	2177	43	10
M (*SD*)	0.33 (0.72)	0.49 (1.10)	0.40 (0.70)
Father absence (*N*)			
Never with father	62	-	-
Father absence before adolescents were 7	239	10	-
Father absence since adolescents were 7	53	-	-
Father presence	1879	28	9
Mother absence (*N*)			
Never with mother	-	-	-
Mother absence before adolescents were 7	-	-	-
Mother absence since adolescents were 7	-	-	-
Mother presence	2215	44	11
Covariates			
Childhood gender nonconforming behavior[f]			
N	1857	33	14
M (*SD*)	37.50 (7.74)	41.71 (9.63)	45.63 (9.60)
Left 2D:4D			
N	2355	50	14
M (*SD*)	0.97 (0.03)	0.97 (0.03)	0.96 (0.02)
Right 2D:4D			
N	2359	50	14
M (*SD*)	0.97 (0.03)	0.97 (0.03)	0.96 (0.02)
Handedness			
N	2172	35	12
M (*SD*)	15.93 (2.84)	15.57 (3.01)	15.58 (2.75)

Note: "-" means 5 or less. Cell counts 5 or less are not presented in order to comply with ALSPAC publication requirements.

[a]The range is from 0 to 16.

[b]The range is from 0 to 15.32.

[c]The range is from 0 to 16.40.

[d]The range is from 0 to 12.25.

[e]The range is from 9 to 45.

[f]The range is from -4.55 to 101.05.

Table3

Univariate Ordered Logistic Regressions for Sexual Orientation Stratified by Sex.

	Boys (*N* = 2349)			Girls (*N* = 2658)		
Variable	OR	OR[a]	OR[b]	OR	OR[a]	OR[b]
Covariates						
Childhood gender nonconforming behavior	0.902***(0.871, 0.935)			1.074***(1.040, 1.108)		1.097***(1.045, 1.151)
Handedness	1.036 (0.931, 1.152)			0.968 (0.881, 1.063)		
Left 2D:4D	1.077* (1.002, 1.157)			1.014 (0.939, 1.094)		
Right 2D:4D		1.185***(1.092, 1.287)	1.245***(1.132,1.368)	0.979 (0.909, 1.056)		
Early life conditions						
Gestational age (Ref = term birth)						
Preterm birth	0.992 (0.311, 3.165)			0.739 (0.179, 3.054)		
Post-term birth	0.936 (0.328, 2.671)			0.575 (0.180, 1.841)		
Birthweight	0.570** (0.382, 0.851)			1.237 (0.761, 2.010)		
Maternal age	0.968 (0.913, 1.027)			1.018 (0.967, 1.072)		
Paternal age	0.985 (0.933, 1.040)			1.016 (0.971, 1.062)		
Prenatal family socioeconomic position	1.059 (0.959, 1.170)			1.110* (1.015, 1.214)		
Number of older brothers		0.902(0.574, 1.417)	1.784 (0.896, 3.553)		1.059 (0.717, 1.565)	1.600 (0.943, 2.716)
Number of older sisters	0.887 (0.559, 1.408)			0.879 (0.564, 1.372)		
Prenatal maternal anxiety/depression	1.063 (0.967, 1.167)			1.077 (0.988, 1.173)		
Parental absence (Ref = parents presence)						
Never with mother or father	3.063* (1.052, 8.920)			2.870 (0.915, 8.997)		
Either parent absence before 7	2.139* (1.050, 4.357)			2.572** (1.309, 5.054)		
Either parent absence since 7	1.354 (0.317, 5.784)			4.135* (1.358, 12.585)		
Duration of breastfeeding before 7	0.921* (0.853, 0.994)			1.043 (0.989, 1.099)		
Postnatal maternal anxiety/depression	1.049 (0.958, 1.150)			1.051 (0.968, 1.142)		
Number of house moves before 7	1.073 (0.896, 1.285)			1.078 (0.898, 1.293)		
Family socioeconomic position before 7	1.000 (0.997, 1.004)			1.001 (0.998, 1.004)		
Number of house moves since 7	1.491*(1.036, 2.146)			1.186 (0.944, 1.492)		
Parent-child relationship	0.971 (0.918, 1.027)			0.931* (0.881, 0.984)		
Pubertal body mass index	1.046 (0.935, 1.170)			1.095*(1.006, 1.192)		
Family socioeconomic position since 7	1.087 (0.947, 1.248)			1.065 (0.938, 1.210)		

Note. We applied *Brant* test to test the proportional odds assumption. If the proportional odds assumption is not violated, we reported one odds ratio in the column OR; if it is violated, we applied the *generalised ordered ordered logit model* (*gologit2*) and reported two odds ratios in the columns OR[a] and OR[b].
[a]Heterosexual boys/girls versus bisexual and homosexual boys/girls.
[b]Heterosexual and bisexual boys/girls versus homosexual boys/girls.
*$p < .05$. **$p < .01$. ***$p < .001$

Table 4.

Three-step Hierarchical Multivariable Ordered Logistic Regressions for Sexual Orientation among Boys (N = 2349)

	Step 1			Step 2			Step 3		
Variable	OR	OR[a]	OR[b]	OR	OR[a]	OR[b]	OR	OR[a]	OR[b]
Covariates									
Childhood gender nonconforming behavior	0.898*** (0.865, 0.932)			0.890*** (0.856, 0.925)			0.888*** (0.853, 0.924)		
Handedness	1.034 (0.922, 1.160)			1.033 (0.920, 1.159)			1.035 (0.920, 1.164)		
Left 2D:4D	0.953 (0.850, 1.070)			0.954 (0.849, 1.072)			0.956 (0.850, 1.076)		
Right 2D:4D		1.213**(1.085, 1.356)	1.273***(1.120, 1.446)		1.224**(1.092, 1.371)	1.283***(1.127, 1.461)		1.219**(1.087, 1.368)	1.278***(1.122, 1.457)
Early life conditions									
Gestational age (Ref = term birth)									
Preterm birth	0.294 (0.067, 1.283)			0.307 (0.068, 1.384)			0.315 (0.070, 1.425)		
Post-term birth	1.233 (0.412, 3.692)			1.229 (0.406, 3.724)			1.305 (0.424, 4.014)		
Birthweight	0.458** (0.261, 0.805)			0.489* (0.276, 0.866)			0.463** (0.259, 0.826)		
Maternal age	0.987 (0.897, 1.087)			0.992 (0.899, 1.094)			1.000 (0.906, 1.105)		
Paternal age	1.013 (0.936, 1.098)			1.017 (0.937, 1.104)			1.019 (0.938, 1.106)		
Prenatal family socioeconomic position	1.045 (0.922, 1.185)			1.063 (0.906, 1.247)			1.060 (0.844, 1.331)		
Number of older brothers		1.078 (0.671, 1.731)	2.069* (1.034, 4.141)		1.117 (0.690, 1.807)	2.176*(1.080, 4.383)		1.145 (0.699, 1.877)	2.254*(1.105, 4.598)
Number of older sisters	0.731 (0.424, 1.261)			0.759 (0.439, 1.313)			0.752 (0.433, 1.307)		
Prenatal maternal anxiety/depression	1.038 (0.937, 1.151)			1.046 (0.903, 1.212)			1.043 (0.899, 1.209)		
Parental absence (Ref = parents presence)									
Never with mother or father	3.336 (0.998, 11.155)			3.708* (1.051, 13.074)			3.449 (0.873, 13.623)		
Either parent absence before 7				2.051 (0.902, 4.664)			1.946 (0.794, 4.771)		
Either parent absence since 7							1.052 (0.218, 5.087)		
Duration of breastfeeding before 7				0.907* (0.836, 0.983)			0.904* (0.834, 0.981)		
Postnatal maternal anxiety/depression				0.994 (0.864, 1.143)			0.994 (0.863, 1.146)		
Number of house moves before 7				1.053 (0.868, 1.278)			1.041 (0.852, 1.272)		
Family socioeconomic position before 7				0.998 (0.993, 1.003)			0.998 (0.993, 1.003)		
Number of house moves since 7							1.360 (0.882, 2.097)		
Parent-child relationship							0.979 (0.922, 1.039)		
Pubertal body mass index							1.079 (0.962, 1.210)		
Family socioeconomic position since 7							0.988 (0.722, 1.352)		

Note. We applied *Brant* test to test the proportional odds assumption. If the proportional odds assumption is not violated, we reported one odds ratio in the column OR; if it is violated, we applied the *generalised ordered ordered logit model* (*gologit2*) and reported two odds ratios in the columns OR[a] and OR[b].
[a]Heterosexual boys versus bisexual and homosexual boys.
[b]Heterosexual and bisexual boys versus homosexual boys.
*$p < .05$. **$p < .01$. ***$p < .00$

Table 5.
Three-step Hierarchical Multivariable Ordered Logistic Regressions for Sexual Orientation among Girls (N = 2658)

	Step 1			Step 2			Step 3		
Variable	OR	OR[a]	OR[b]	OR	OR[a]	OR[b]	OR	OR[a]	OR[b]
Covariates									
Childhood gender nonconforming behavior		1.074***(1.038, 1.110)	1.096**(1.038, 1.157)		1.074***(1.039, 1.111)	1.097**(1.039, 1.158)		1.072***(1.036, 1.110)	1.095**(1.035, 1.157)
Handedness	0.979 (0.888, 1.079)			0.977 (0.885, 1.078)			0.968 (0.873, 1.073)		
Left 2D:4D	1.051 (0.943, 1.170)			1.053 (0.945, 1.173)			1.057 (0.948, 1.180)		
Right 2D:4D	0.958 (0.861, 1.066)			0.959 (0.861, 1.067)			0.950 (0.851, 1.061)		
Early life conditions									
Gestational age (Ref = term birth)									
Preterm birth	1.158 (0.248, 5.412)			1.220 (0.256, 5.813)			1.101 (0.218, 5.553)		
Post-term birth	0.617 (0.187, 2.033)			0.623 (0.188, 2.064)			0.663 (0.195, 2.250)		
Birthweight	1.393 (0.818, 2.374)			1.416 (0.824, 2.433)			1.376 (0.795, 2.380)		
Maternal age	1.050 (0.966, 1.140)			1.039 (0.955, 1.130)			1.049 (0.963, 1.143)		
Paternal age	1.006 (0.941, 1.075)			1.012 (0.946, 1.082)			1.014 (0.946, 1.087)		
Prenatal family socioeconomic position	1.126* (1.011, 1.254)			1.149* (1.006, 1.311)			1.343** (1.109, 1.627)		
Number of older brothers		0.771 (0.497, 1.196)	1.162 (0.667, 2.025)		0.727 (0.465, 1.136)	1.095 (0.624, 1.922)		0.698 (0.443, 1.098)	1.055 (0.600, 1.855)
Number of older sisters	0.808 (0.505, 1.292)			0.785 (0.488, 1.262)			0.756 (0.465, 1.229)		
Prenatal maternal anxiety/depression	1.035 (0.943, 1.136)			1.031 (0.909, 1.170)			1.027 (0.904, 1.166)		
Parental absence (Ref = parents presence)									
Never with mother or father	2.002 (0.593, 6.755)			1.584 (0.419, 5.991)			2.620 (0.561, 12.248)		
Either parent absence before 7				2.501* (1.201, 5.212)			2.990** (1.361, 6.570)		
Either parent absence since 7							4.494* (1.368, 14.760)		
Duration of breastfeeding before 7				1.056 (0.997, 1.119)			1.063* (1.001, 1.129)		
Postnatal maternal anxiety/depression				0.998 (0.885, 1.126)			1.003 (0.887, 1.135)		
Number of house moves before 7				1.062 (0.867, 1.300)			1.055 (0.853, 1.305)		
Family socioeconomic position before 7				1.000 (0.996, 1.004)			1.000 (0.996, 1.005)		
Number of house moves since 7							1.161 (0.865, 1.558)		
Parent-child relationship							0.928* (0.873, 0.986)		
Pubertal body mass index							1.078 (0.978, 1.189)		
Family socioeconomic position since 7							0.744* (0.564, 0.981)		

Note. We applied *Brant* test to test the proportional odds assumption. If the proportional odds assumption is not violated, we reported one odds ratio in the column OR; if it is violated, we applied the *generalised ordered ordered logit model* (*gologit2*) and reported two odds ratios in the columns OR[a] and OR[b].

[a]Heterosexual girls versus bisexual and homosexual girls.

[b]Heterosexual and bisexual girls versus homosexual girls.

*$p < .05$. **$p < .01$. ***$p < .001$.

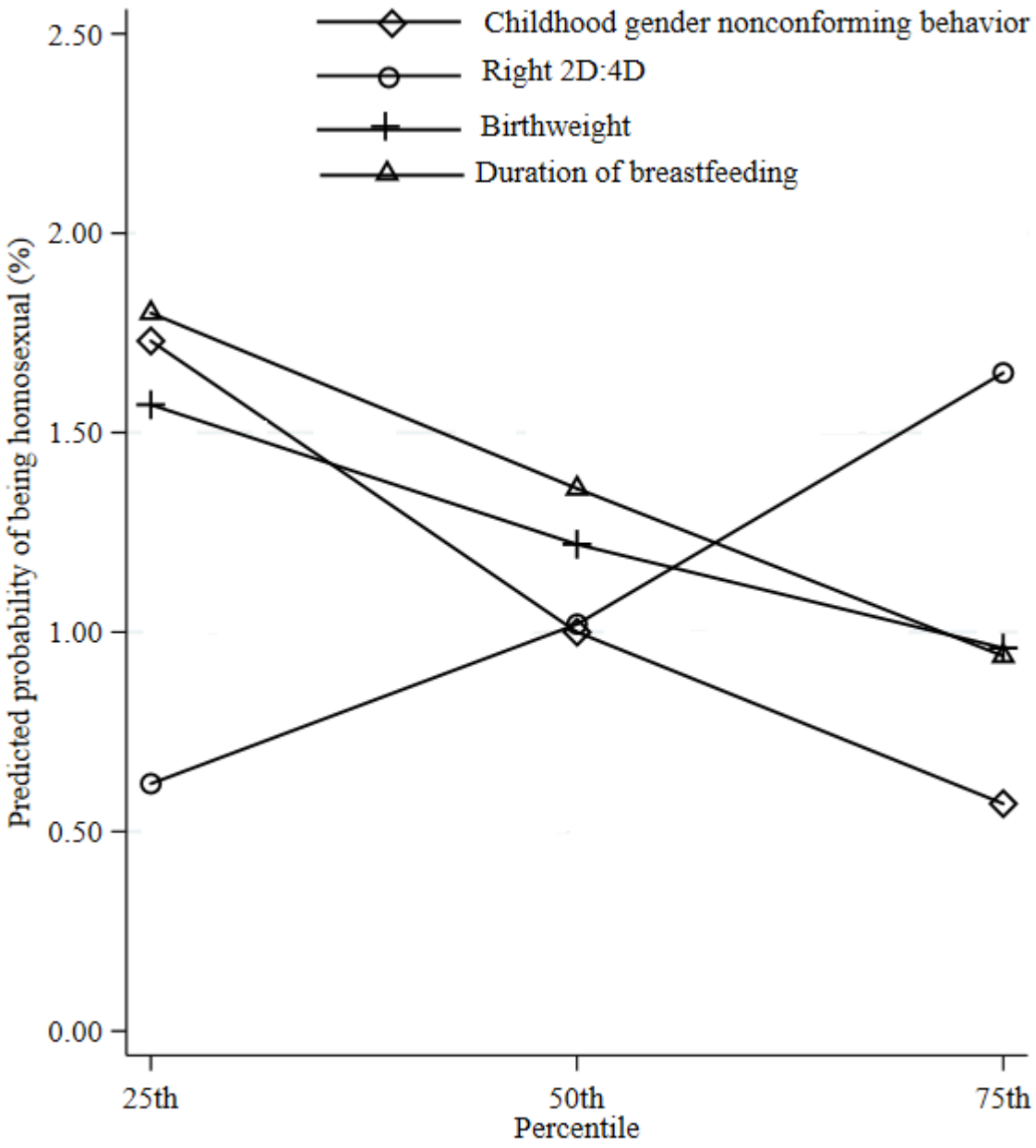

Figure 1. Predicted probability of being homosexual for significant early life factors and covariates in the final model among boys.

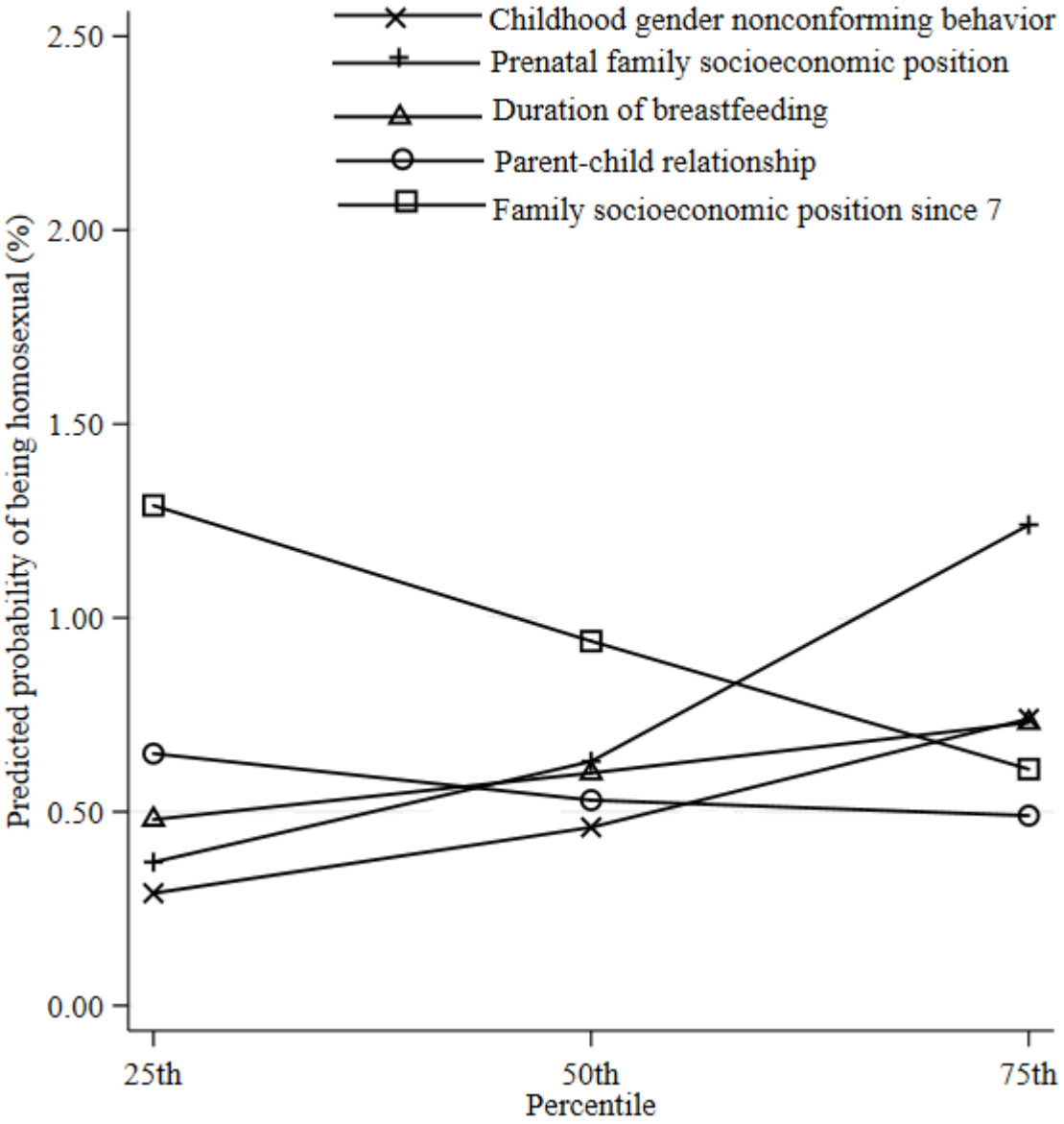

Figure 2. Predicted probability of being homosexual for significant continuous early life factors and covariates in the final model among girls.

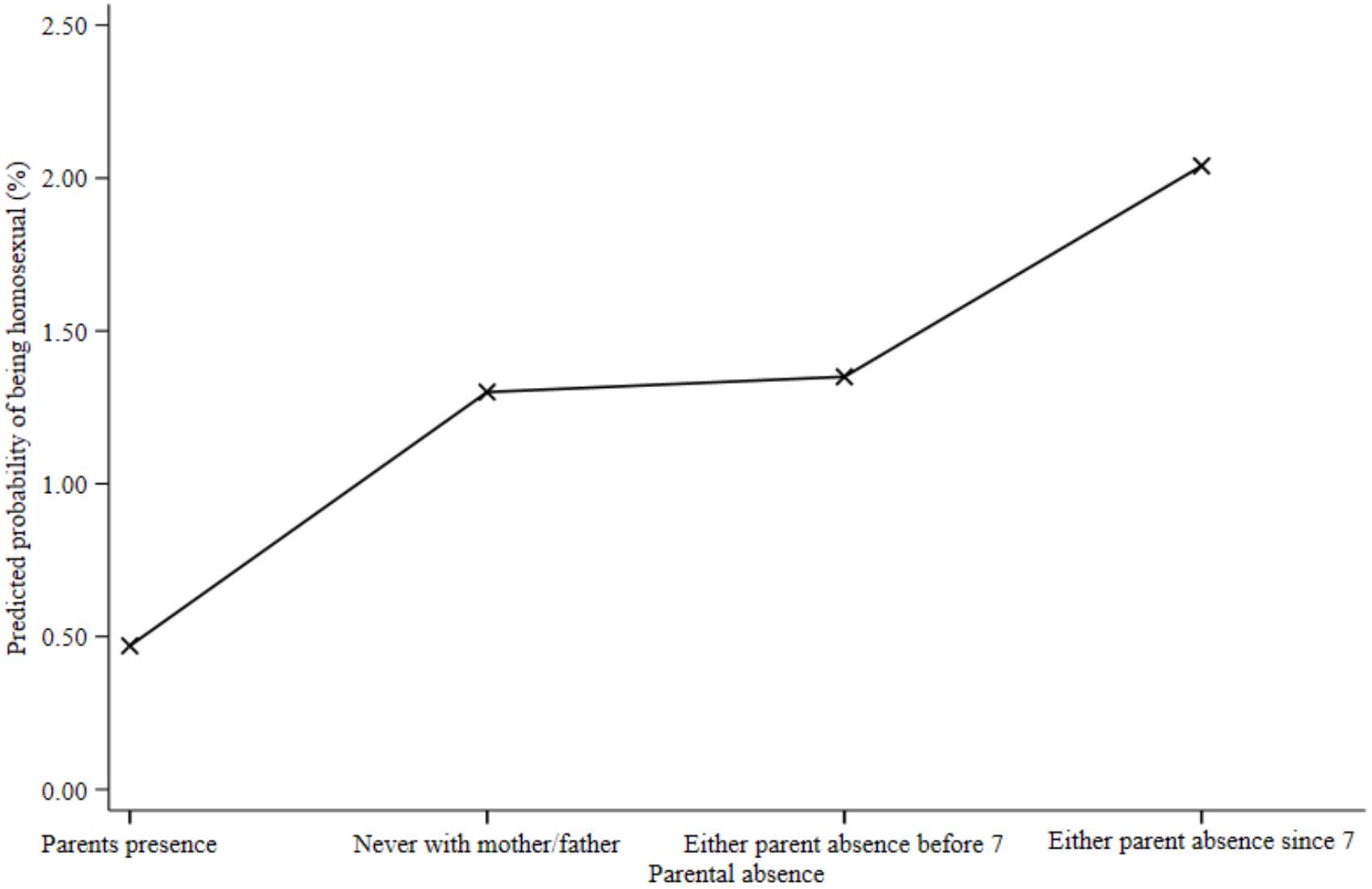

Figure 3. Predicted probability of being homosexual for parental absence in the final model among girls

Supplemental Text 1

Sexual behavior

When adolescents were 15.5 years old, they were required to report whether they had engaged in fourteen sexual activities from the Adolescent Sexual Activities Index (Hansen, Paskett, & Carter, 1999). Those sexual activities were presented in order from low (e.g., hug or hold hand) to high (e.g., have oral sex or have sexual intercourse) intensity. Adolescents were required to report whether they had engaged in each sexual activity in the past year, and the sex of the person with whom they engaged in each activity. Adolescents who reported not having engaged in a particular sexual activity received a score of 0 on that activity, those who reported engaging in the activity with opposite sex received a score of 1 on that activity, and those who reported engaging in the activity with the same-sex or both sexes received a score of 2 on that activity. The total score was used in the multiple imputation, with a higher score indicating more same-sex sexual activities. ASAI is a validated self-report measure and has high internal consistency (Cronbach's $\alpha = .93$) (Hansen et al., 1999).

Reference

Hansen, W. B., Paskett, E. D., & Carter, L. J. (1999). The Adolescent Sexual Activity Index (ASAI): A standardized strategy for measuring interpersonal heterosexual behaviors among youth. *Health Education Research, 14*, 485-490.

Supplemental Table

Supplemental Table 1. *Exploratory Factor Analysis of Parent-Child-Relationship and Model Fit Statistics*

Item	Factor loading	Model fit statistics
Study child is understood by parents	0.59	CFI= 0.92
Study child likes their parents	0.54	TLI= 0.90
Study child is liked by parents	0.51	RMSEA = .05
Study child wants to bring own children up same way as they have been brought up	0.42	90%CI = (.04, .05)
Parents and study child spend a lot of time together	0.60	SRMR= .04
Study child's parents is easy to talk to	0.70	
Study child get along well with parents	0.77	
Study child and parents have a lot of fun together	0.72	
Study child's parents usually unhappy/disappointed with what they do	-0.39	

Supplemental Table 2. *Results of Principal Component Analysis for Family Socioeconomic Position*

	Factor loading
Prenatal family socioeconomic position	
Mother's education	0.52
Father's education	0.52
Mother's occupation	0.45
Father's occupation	0.42
Financial difficulties	0.28
Family socioeconomic position before adolescents were 7	
Mother's education	0.46
Father's education	0.45
Mother's occupation	0.41
Father's occupation	0.37
Financial difficulties	0.32
Household income	0.43
Family socioeconomic position since adolescents were 7	
Mother's education	0.52
Father's education	0.54
Financial difficulties	0.39
Household income	0.54

Supplemental Table 3. *Logistic Regressions for Sexual Orientation among Boys (N = 2349)*

Variable	Univariate logistic regression[a]	Three-step hierarchical multivariable logistic regression[a]		
Covariates				
Childhood gender nonconforming behavior	0.899*** (0.866, 0.933)	0.896***(0.862, 0.932)	0.890*** (0.855, 0.926)	0.888*** (0.852, 0.925)
Handedness	1.036 (0.932, 1.153)	1.035 (0.923, 1.160)	1.033 (0.921, 1.160)	1.036 (0.921, 1.166)
Left 2D:4D	1.077* (1.002, 1.157)	0.955 (0.850, 1.072)	0.954 (0.848, 1.073)	0.956 (0.849, 1.076)
Right 2D:4D	1.186***(1.092, 1.288)	1.207**(1.079, 1.350)	1.215** (1.084, 1.362)	1.212** (1.079, 1.360)
Early life conditions				
Gestational age (Ref = term birth)				
Preterm birth	0.986 (0.310, 3.144)	0.298 (0.069, 1.295)	0.310 (0.070, 1.381)	0.318 (0.071, 1.423)
Post-term birth	0.940 (0.330, 2.681)	1.226 (0.412, 3.651)	1.231 (0.409, 3.704)	1.304 (0.427, 3.981)
Birthweight	0.570 **(0.382, 0.851)	0.465**(0.264, 0.818)	0.496* (0.280, 0.880)	0.469* (0.262, 0.839)
Maternal age	0.968 (0.912 1.027)	0.988 (0.898, 1.087)	0.992 (0.899, 1.094)	1.000 (0.906, 1.104)
Paternal age	0.985 (0.932, 1.040)	1.010 (0.933, 1.095)	1.013 (0.934, 1.100)	1.015 (0.935, 1.102)
Prenatal family socioeconomic position	1.059 (0.959, 1.170)	1.044 (0.920, 1.184)	1.059 (0.904, 1.240)	1.051 (0.839, 1.316)
Number of older brothers	0.905 (0.576, 1.424)	1.087 (0.676, 1.748)	1.127 (0.696, 1.826)	1.161 (0.708, 1.903)
Number of older sisters	0.890 (0.560, 1.412)	0.743 (0.432, 1.279)	0.772 (0.447, 1.333)	0.770 (0.444, 1.334)
Prenatal maternal anxiety/depression	1.062 (0.967, 1.166)	1.034 (0.932, 1.146)	1.036 (0.895, 1.200)	1.031 (0.889, 1.196)
Parental absence (Ref = parents presence)				
Never with mother or father	3.121* (1.070, 9.101)	3.469* (1.037, 11.606)	3.829* (1.091, 13.438)	3.503 (0.894, 13.715)
Either parent absence before 7	2.163* (1.061, 4.406)		2.087 (0.917, 4.751)	1.977 (0.808, 4.836)
Either parent absence since 7	1.355 (0.318, 5.783)			0.988 (0.199, 4.914)
Duration of breastfeeding before 7	0.921* (0.854, 0.994)		0.911* (0.840, 0.987)	0.909* (0.838, 0.985)
Postnatal maternal anxiety/depression	1.048 (0.957, 1.148)		1.000 (0.870, 1.149)	1.001 (0.869, 1.152)
Number of house moves before 7	1.073 (0.896, 1.285)		1.046 (0.861, 1.270)	1.033 (0.844, 1.264)
Family socioeconomic position before 7	1.000 (0.997, 1.004)		0.998 (0.993, 1.003)	0.998 (0.993, 1.003)
Number of house moves since 7	1.498* (1.040, 2.158)			1.361 (0.879, 2.107)
Parent-child relationship	0.971 (0.919, 1.027)			0.979 (0.923, 1.040)
Pubertal body mass index	1.047 (0.936, 1.171)			1.081 (0.965, 1.212)
Family socioeconomic position since 7	1.088 (0.948, 1.248)			0.997 (0.733, 1.356)

Note. [a]Heterosexual boys are the reference group.

*$p < .05$. **$p < .01$. ***$p < .001$.

Supplemental Table 4. *Logistic Regressions for Sexual Orientation among Girls (N = 2658)*

Variable	Univariate logistic regression[a]	Three-step hierarchical multivariable logistic regression[a]		
Covariates				
Childhood gender nonconforming behavior	1.074*** (1.040, 1.109)	1.074*** (1.039, 1.111)	1.074*** (1.039, 1.111)	1.072*** (1.036, 1.110)
Handedness	0.968 (0.880, 1.063)	0.979 (0.888, 1.080)	0.977 (0.885, 1.079)	0.968 (0.873, 1.073)
Left 2D:4D	1.014 (0.940, 1.095)	1.052 (0.944, 1.171)	1.054 (0.946, 1.174)	1.059 (0.949, 1.181)
Right 2D:4D	0.980 (0.909, 1.056)	0.958 (0.861, 1.066)	0.958 (0.861, 1.067)	0.950 (0.851, 1.061)
Early life conditions				
Gestational age (Ref = term birth)				
Preterm birth	0.734 (0.178, 3.030)	1.141 (0.243, 5.351)	1.187 (0.247, 5.705)	1.046 (0.202, 5.430)
Post-term birth	0.578 (0.180, 1.849)	0.623 (0.189, 2.054)	0.630 (0.190, 2.089)	0.674 (0.199, 2.282)
Birthweight	1.237 (0.761, 2.010)	1.400 (0.821, 2.390)	1.420 (0.824, 2.445)	1.378 (0.795, 2.389)
Maternal age	1.018 (0.967, 1.073)	1.051 (0.968, 1.142)	1.041 (0.956, 1.133)	1.051 (0.965, 1.146)
Paternal age	1.015 (0.971, 1.062)	1.005 (0.940, 1.074)	1.010 (0.944, 1.080)	1.012 (0.944, 1.084)
Prenatal family socioeconomic position	1.110* (1.014, 1.214)	1.125* (1.011, 1.253)	1.148* (1.006, 1.310)	1.339** (1.104, 1.624)
Number of older brothers	1.065 (0.715, 1.586)	0.774 (0.494, 1.214)	0.732 (0.464, 1.156)	0.703 (0.444, 1.114)
Number of older sisters	0.879 (0.563, 1.372)	0.809 (0.505, 1.294)	0.787 (0.489, 1.267)	0.761 (0.468, 1.237)
Prenatal maternal anxiety/depression	1.078 (0.989, 1.174)	1.037 (0.945, 1.139)	1.032 (0.909, 1.172)	1.029 (0.906, 1.169)
Parental absence (Ref = parents presence)				
Never with mother or father	2.895 (0.927, 9.039)	2.011 (0.599, 6.751)	1.600 (0.428, 5.980)	2.664 (0.584, 12.145)
Either parent absence before 7	2.583** (1.314, 5.077)		2.506* (1.203, 5.219)	3.000** (1.360, 6.616)
Either parent absence since 7	4.199* (1.393, 12.659)			4.611* (1.431, 14.856)
Duration of breastfeeding before 7	1.042 (0.988, 1.099)		1.056 (0.997, 1.118)	1.062* (1.000, 1.128)
Postnatal maternal anxiety/depression	1.052 (0.968, 1.143)		1.000 (0.886, 1.130)	1.005 (0.888, 1.138)
Number of house moves before 7	1.077 (0.898, 1.293)		1.059 (0.865, 1.296)	1.051 (0.851, 1.298)
Family socioeconomic position before 7	1.001 (0.998, 1.004)		1.000 (0.996, 1.004)	1.000 (0.996, 1.004)
Number of house moves since 7	1.188 (0.945, 1.493)			1.165 (0.868, 1.562)
Parent-child relationship	0.931* (0.881, 0.984)			0.928* (0.873, 0.987)
Pubertal body mass index	1.095* (1.006, 1.191)			1.077 (0.978, 1.186)
Family socioeconomic position since 7	1.065 (0.937, 1.210)			0.747* (0.566, 0.986)

Note. [a]Heterosexual girls are the reference group.

*$p < .05$. **$p < .01$. ***$p < .001$.

Supplemental Table 5. *Multinomial Logistic Regressions for Sexual Orientation among Boys (N = 2349)*

	Univariate multinomial logistic regression[a]		Three-step hierarchical multivariable multinomial logistic regression[a]					
Variable	Bisexual	Homosexual	Bisexual	Homosexual	Bisexual	Homosexual	Bisexual	Homosexual
Covariates								
Childhood gender nonconforming behavior	0.918** (0.873, 0.966)	0.882*** (0.837, 0.929)	0.919** (0.872, 0.969)	0.870*** (0.823, 0.921)	0.915** (0.867, 0.966)	0.860*** (0.809, 0.913)	0.912** (0.863, 0.964)	0.859*** (0.807, 0.913)
Handedness	1.080 (0.912, 1.279)	1.007 (0.877, 1.156)	1.078 (0.901, 1.289)	1.006 (0.865, 1.169)	1.079 (0.901, 1.291)	1.001 (0.858, 1.168)	1.083 (0.902, 1.300)	1.003 (0.856, 1.175)
Left 2D:4D	1.032 (0.921, 1.157)	1.109* (1.014, 1.212)	0.962 (0.823, 1.125)	0.943 (0.796, 1.116)	0.961 (0.821, 1.123)	0.938 (0.788, 1.118)	0.961 (0.822, 1.124)	0.941 (0.786, 1.127)
Right 2D:4D	1.078 (0.957, 1.215)	1.294***(1.153, 1.453)	1.097 (0.942, 1.277)	1.336** (1.131, 1.579)	1.101 (0.944, 1.284)	1.365***(1.146,1.625)	1.102 (0.946, 1.285)	1.358** (1.134, 1.626)
Early life conditions								
Gestational age (Ref = term birth)								
Preterm birth	0.574 (0.077, 4.271)	1.371 (0.331, 5.681)	0.158 (0.016, 1.580)	0.531 (0.074, 3.831)	0.162 (0.016, 1.652)	0.582 (0.076, 4.454)	0.154 (0.015, 1.598)	0.631 (0.080, 4.992)
Post-term birth	1.340 (0.400, 4.485)	0.526 (0.068, 4.088)	1.624 (0.462, 5.705)	0.762 (0.090, 6.446)	1.590 (0.449, 5.628)	0.812 (0.093, 7.076)	1.731 (0.478, 6.269)	0.864 (0.096, 7.779)
Birthweight	0.616 (0.353, 1.075)	0.531* (0.302, 0.934)	0.426*(0.197, 0.920)	0.538 (0.238, 1.216)	0.437* (0.201, 0.946)	0.611 (0.262, 1.430)	0.399* (0.184, 0.861)	0.601 (0.246, 1.463)
Maternal age	0.963 (0.887, 1.044)	0.974 (0.894, 1.060)	1.005 (0.883, 1.144)	0.968 (0.834, 1.124)	1.008 (0.882, 1.152)	0.974 (0.837, 1.134)	1.017 (0.889, 1.164)	0.984 (0.842, 1.149)
Paternal age	0.967 (0.894, 1.045)	1.001 (0.927, 1.079)	0.989 (0.882, 1.109)	1.032 (0.918, 1.160)	0.989 (0.881, 1.110)	1.038 (0.918, 1.174)	0.991 (0.883, 1.113)	1.038 (0.917, 1.175)
Prenatal family socioeconomic position	1.026 (0.895, 1.175)	1.090 (0.940, 1.265)	0.987 (0.835, 1.166)	1.101 (0.910, 1.332)	0.983 (0.798, 1.212)	1.141 (0.892, 1.460)	0.927 (0.693, 1.240)	1.193 (0.834, 1.709)
Number of older brothers	0.461 (0.191, 1.111)	1.318 (0.785, 2.213)	0.588 (0.241, 1.435)	1.580 (0.873, 2.858)	0.599 (0.245, 1.464)	1.672 (0.896, 3.120)	0.624 (0.253, 1.544)	1.683 (0.891, 3.181)
Number of older sisters	1.152 (0.662, 2.006)	0.624 (0.279, 1.396)	1.124 (0.596, 2.121)	0.395 (0.148, 1.050)	1.154 (0.609, 2.186)	0.414 (0.152, 1.126)	1.175 (0.615, 2.246)	0.413 (0.151, 1.127)
Prenatal maternal anxiety/depression	1.023 (0.894, 1.170)	1.098 (0.966, 1.248)	0.984 (0.851, 1.137)	1.104 (0.956, 1.275)	1.017 (0.825, 1.254)	1.077 (0.881, 1.317)	1.007 (0.815, 1.245)	1.080 (0.877, 1.329)
Parental absence (Ref = parents presence)								
Never with mother or father	5.516** (1.562, 19.478)	1.366 (0.178, 10.477)	6.664** (1.602, 27.727)	1.122 (0.115, 10.957)	7.120** (1.634, 31.025)	1.148 (0.098, 13.447)	5.816* (1.160, 29.168)	1.190 (0.087, 16.222)
Either parent absence before 7	3.596**(1.470, 8.795)	1.135 (0.335, 3.846)			3.917** (1.432, 10.713)	0.896 (0.216, 3.713)	3.431* (1.178, 9.993)	0.936 (0.203, 4.324)
Either parent absence since 7	1.527 (0.200, 11.676)	1.205 (0.159, 9.109)					1.014 (0.122, 8.420)	0.877 (0.083, 9.308)
Duration of breastfeeding before 7	0.952 (0.863, 1.050)	0.886[b] (0.783, 1.002)			0.952 (0.858, 1.057)	0.858* (0.749, 0.982)	0.950 (0.856, 1.054)	0.857* (0.747, 0.982)
Postnatal maternal anxiety/depression	0.991 (0.862, 1.140)	1.096 (0.972, 1.236)			0.956 (0.774, 1.181)	1.034 (0.862, 1.241)	0.958 (0.771, 1.189)	1.032 (0.856, 1.244)
Number of house moves before 7	1.087 (0.844, 1.400)	1.048 (0.794, 1.385)			0.981 (0.748, 1.288)	1.120 (0.819, 1.532)	0.964 (0.727, 1.279)	1.116 (0.808, 1.541)
Family socioeconomic position before 7	1.000 (0.996, 1.005)	1.000 (0.995, 1.005)			0.999 (0.993, 1.006)	0.997 (0.989, 1.004)	0.999 (0.993, 1.006)	0.997 (0.989, 1.005)
Number of house moves since 7	1.710* (1.076, 2.717)	1.279 (0.712, 2.296)					1.539 (0.898, 2.638)	1.175 (0.566, 2.437)

To be continued

	Univariate multinomial logistic regression[a]		Three-step hierarchical multivariable multinomial logistic regression[a]					
Variable	Bisexual	Homosexual	Bisexual	Homosexual	Bisexual	Homosexual	Bisexual	Homosexual
Early life conditions								
Parent-child relationship	0.998 (0.909, 1.095)	0.954 (0.890, 1.022)					1.001 (0.906, 1.106)	0.969 (0.896, 1.047)
Pubertal body mass index	1.097 (0.959, 1.255)	0.987 (0.824, 1.183)					1.127 (0.976, 1.302)	1.037 (0.858, 1.252)
Family socioeconomic position since 7	1.117 (0.940, 1.326)	1.058 (0.853, 1.311)					1.072 (0.761, 1.511)	0.911 (0.531, 1.562)

Note. [a]Heterosexual boys are the reference group.

[b]p =.053.

*p < .05. **p < .01. ***p < .001.

Supplemental Table 6. *Multinomial Logistic Regressions for Sexual Orientation among Girls (N = 2658)*

	Univariate multinomial logistic regression[a]		Three-step hierarchical multivariable multinomial logistic regression[a]					
Variable	Bisexual	Homosexual	Bisexual	Homosexual	Bisexual	Homosexual	Bisexual	Homosexual
Covariates								
Childhood gender nonconforming behavior	1.063** (1.025, 1.103)	1.111** (1.045, 1.182)	1.065** (1.025, 1.107)	1.112** (1.038, 1.191)	1.065** (1.025, 1.107)	1.117** (1.039, 1.201)	1.063** (1.022, 1.106)	1.117** (1.035, 1.206)
Handedness	0.968 (0.869, 1.079)	0.970 (0.813, 1.156)	0.979 (0.876, 1.095)	0.984 (0.814, 1.190)	0.978 (0.873, 1.095)	0.983 (0.806, 1.199)	0.970 (0.865, 1.089)	0.967 (0.779, 1.200)
Left 2D:4D	1.046 (0.960, 1.141)	0.914 (0.778, 1.073)	1.082 (0.957, 1.224)	0.949 (0.740, 1.216)	1.085 (0.960, 1.227)	0.942 (0.733, 1.211)	1.091 (0.964, 1.234)	0.931 (0.715, 1.212)
Right 2D:4D	1.006 (0.924, 1.095)	0.895 (0.760, 1.052)	0.964 (0.854, 1.088)	0.947 (0.740, 1.211)	0.963 (0.853, 1.088)	0.957 (0.745, 1.230)	0.955 (0.843, 1.082)	0.957 (0.736, 1.243)
Early life conditions								
Gestational age (Ref = term birth)								
Preterm birth	0.450 (0.062, 3.290)	1.626 (0.213, 12.386)	0.669 (0.082, 5.451)	2.806 (0.260, 30.347)	0.677 (0.082, 5.604)	3.272 (0.271, 39.450)	0.624 (0.073, 5.360)	2.541 (0.157, 41.205)
Post-term birth	0.750 (0.232, 2.423)	-	0.781 (0.232, 2.626)	-	0.781 (0.230, 2.646)	-	0.829 (0.240, 2.859)	-
Birthweight	1.232 (0.710, 2.139)	1.256 (0.455, 3.464)	1.375 (0.746, 2.536)	1.536 (0.505, 4.675)	1.383 (0.743, 2.574)	1.643 (0.524, 5.148)	1.346 (0.719, 2.518)	1.607 (0.487, 5.302)
Maternal age	1.026 (0.967, 1.089)	0.993 (0.892, 1.106)	1.087 (0.989, 1.194)	0.944 (0.797, 1.117)	1.076 (0.977, 1.185)	0.935 (0.786, 1.112)	1.086 (0.984, 1.199)	0.942 (0.785, 1.130)
Paternal age	1.008 (0.956, 1.063)	1.038 (0.947, 1.137)	0.984 (0.909, 1.066)	1.061 (0.933, 1.208)	0.987 (0.911, 1.070)	1.073 (0.939, 1.226)	0.988 (0.910, 1.073)	1.080 (0.939, 1.243)
Prenatal family socioeconomic position	1.083 (0.977, 1.201)	1.198 (0.995, 1.442)	1.098 (0.971, 1.242)	1.217 (0.969, 1.529)	1.134 (0.973, 1.321)	1.206 (0.912, 1.593)	1.306* (1.039, 1.640)	1.486 (0.973, 2.269)
Number of older brothers	0.817 (0.493, 1.352)	1.770*(1.003, 3.125)	0.584 (0.336, 1.013)	1.370 (0.663, 2.833)	0.570* (0.326, 0.996)	1.144 (0.522, 2.508)	0.547* (0.309, 0.968)	1.154 (0.516, 2.582)
Number of older sisters	0.876 (0.528, 1.452)	0.881 (0.364, 2.131)	0.791 (0.464, 1.349)	0.863 (0.343, 2.170)	0.786 (0.458, 1.347)	0.762 (0.292, 1.989)	0.764 (0.441, 1.323)	0.691 (0.252, 1.892)
Prenatal maternal anxiety/depression	1.100 (1.000, 1.210)	1.000 (0.824, 1.213)	1.067 (0.962, 1.183)	0.935 (0.755, 1.158)	1.056 (0.917, 1.217)	0.951 (0.720, 1.255)	1.052 (0.913, 1.213)	0.947 (0.710, 1.265)
Parental absence (Ref = parents presence)								
Never with mother or father	3.586*(1.108, 11.609)	-	2.565 (0.727, 9.047)	-	2.159 (0.564, 8.267)	-	3.448 (0.758, 15.681)	-
Either parent absence before 7	2.937**(1.372, 6.288)	1.607 (0.352, 7.326)			3.037**(1.346, 6.853)	1.116 (0.191, 6.506)	3.571**(1.509, 8.447)	1.467 (0.221, 9.742)
Either parent absence since 7	5.431**(1.873, 15.750)	-					6.297** (1.998,19.842)	-
Duration of breastfeeding before 7	1.031 (0.968, 1.097)	1.079 (0.967, 1.205)			1.041 (0.974, 1.113)	1.109 (0.983, 1.250)	1.047 (0.977, 1.123)	1.116 (0.982, 1.268)
Postnatal maternal anxiety/depression	1.074 (0.980, 1.177)	0.968 (0.797, 1.177)			1.011 (0.882, 1.159)	0.962 (0.741, 1.250)	1.017 (0.885, 1.168)	0.958 (0.729, 1.259)
Number of house moves before 7	1.057 (0.852, 1.313)	1.116 (0.790, 1.576)			1.023 (0.809, 1.295)	1.169 (0.792, 1.726)	1.014 (0.793, 1.298)	1.164 (0.777, 1.745)
Family socioeconomic position before 7	1.000 (0.996, 1.004)	1.005 (0.999, 1.012)			0.999 (0.994, 1.004)	1.004 (0.995, 1.012)	0.999 (0.994, 1.004)	1.004 (0.995, 1.013)
Number of house moves since 7	1.202 (0.945, 1.529)	1.072 (0.550, 2.088)					1.162 (0.839, 1.608)	1.138 (0.547, 2.367)
Parent-child relationship	0.940 (0.879, 1.005)	0.907* (0.829, 0.992)					0.941 (0.873, 1.013)	0.891* (0.800, 0.993)

To be continued

	Univariate multinomial logistic regression[a]		Three-step hierarchical multivariable multinomial logistic regression[a]					
Variable	Bisexual	Homosexual	Bisexual	Homosexual	Bisexual	Homosexual	Bisexual	Homosexual
Early life conditions								
Pubertal body mass index	1.084 (0.985, 1.192)	1.115 (0.928, 1.340)					1.073 (0.964, 1.194)	1.083 (0.862, 1.359)
Family socioeconomic position since 7	1.058 (0.917, 1.220)	1.086 (0.842, 1.401)					0.767 (0.561, 1.050)	0.664 (0.368, 1.198)

Note. [a]Heterosexual girls are the reference group.

No risk ratios were reported when the cell size were less than 5.

*p < .05. **p < .01. ***p < .001.